SUPPLEMENT ARTICLE

Characterization of Rotavirus Strains Detected in Windhoek, Namibia during 1998–1999

Nicola Page,[1,2] Cara Pager,[2] and A. Duncan Steele[2,3]

[1]Viral Gastroenteritis Unit, National Institute for Communicable Diseases, Johannesburg, and [2]MRC Diarrhoeal Pathogens Research Unit, University of Limpopo Medunsa Campus, Pretoria, South Africa; and [3]Department of Immunizations, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland

Background. Namibia, located on the southwestern coast of southern Africa, is characterized by vast deserts, limited fresh water, and low population density. Mortality estimates among children <5 of age are 63 deaths per 1000 live-births, with diarrheal diseases contributing to 3% of these deaths. Data on the burden of rotavirus disease and circulating serotypes in Namibia are currently not available.

Materials and methods. From May 1998 through December 1999, 815 stool specimens were collected from children <5 years of age who attended the Windhoek State Hospital, Windhoek, Namibia, for diarrhea. Specimens were screened for the presence of rotavirus antigens. Rotavirus-positive specimens were further analyzed to determine electropherotype, subgroup (SG) specificity, and G and P genotypes.

Results. Rotavirus was detected in 113 (13.8%) of 815 specimens, with the majority of infections occurring in children <18 months of age. Strains bearing 1 long electropherotype, SGII, and G1P[8] or G1P[6] specificity predominated during the 20-month study period. In addition to the typical winter rotavirus season, a peak in rotavirus infection was also observed during the summer.

Conclusions. Serotypes G1P[8], G1P[6], G1P[4], and G2P[4] were found throughout the study period, predominantly in children <18 months of age. The observed summer rotavirus peak coincided with increased rainfall in Namibia and an increase in the diversity of detected serotypes. During the October to December 1999 peak, 2 G9P[6] strains and 1 G8P[4] strain were identified. Expanded and updated information on prevalence of rotavirus infection, circulating serotypes, and burden of disease will be required to enable local government to make decisions on the implementation of rotavirus vaccination in Namibia.

Diarrheal diseases, exacerbated by pediatric malnutrition and poor water, sanitation, and hygiene, are the second most common diseases in infants and young children in developing regions [1]. Diarrhea is also one of the leading causes of death among children in developing countries, accounting for a mean of 21% of all deaths among children <5 years of age and 1.6–2.5 million deaths annually [2].

Rotavirus is the foremost etiological agent of gastroenteritis, causing 20%–30% of childhood diarrheal episodes [3]. A recent estimate of diarrhea-related childhood deaths from rotavirus infection is 611,000 deaths per year (range, 454,000–705,000 death per year) [4]. Efforts to improve sanitation and provide clean water have not decreased the high incidence of rotavirus infection in developed countries, focusing the need to develop an effective vaccine intervention as the first strategy of prevention [5].

Rotavirus possesses 11 distinct segments of double-stranded RNA (dsRNA) enclosed in 3 protein layers and is classified according to 3 antigenic markers (VP6, VP4, and VP7). The VP6 protein forms the inner capsid of the particle and specifies the major determinants of rotavirus serogroup and subgroup reactivity [6]. The VP7 and VP4 proteins form the smooth outer capsid (G serotype) and short spike (P genotype), respectively, and are the major antigens inducing neutralizing immune responses in human infection. At least 11 G serotype and 11 P genotype group A rotavirus strains

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Reprints or correspondence: Dr Nicola Page, Private Bag x4, Sandringham, Gauteng 2131, South Africa (nicolap@nicd.ac.za).

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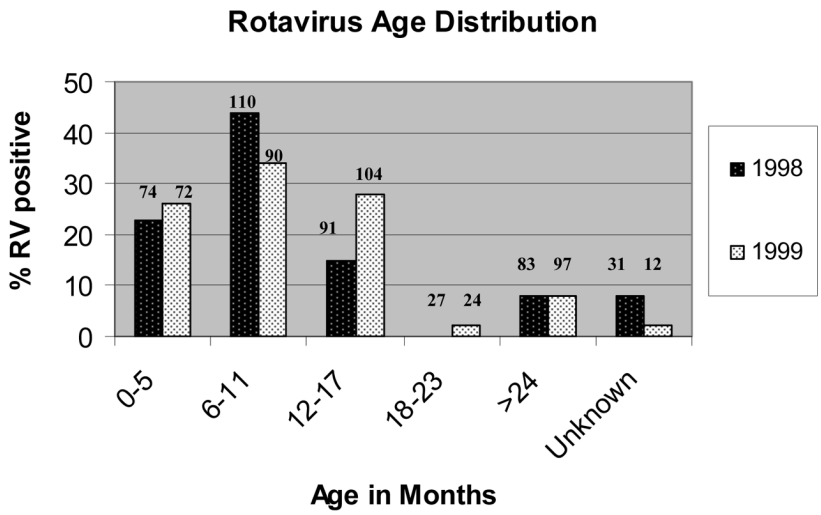

Figure 1. Percentage rotavirus-positive specimens ($n = 113$), by age group, collected during May 1998–December 1999 at the Windhoek State Hospital in Namibia. Numbers above the bars indicate the number of stool specimens tested for each age range per year.

have been identified in humans [6], although serotypes G1P[8], G2P[4], G3P[8], and G4P[8] are thought to be of major epidemiological importance because of their worldwide distribution [7–9]. Serotype G9P[8] has emerged in recent years and is currently acknowledged to be the fifth most important circulating serotype [8, 9], in addition to regionally significant serotypes, including G8 and P[6] in Africa [8, 10].

Namibia spans 825,418 km^2 with a population of ~2 million and is located on the west coast of southern Africa. The country is characterized by vast deserts, limited fresh water, and low population density in widely separated human settlements. The available estimated mortality among Namibian children <5 of age is 63 deaths per 1000 live births, with diarrheal diseases contributing to 3% of deaths [11]. To date, only a single study examining seroepidemiology of calicivirus has been conducted in Namibia [12], and data on the burden of rotavirus disease and circulating serotypes are nonexistent. This study reports the detection and characterization of rotavirus strains collected from the Windhoek State Hospital from May 1998 through December 1999.

MATERIALS AND METHODS

From May 1998 through December 1999, 815 stool specimens were collected from infants and young children <5 years of age who either presented with diarrhea or were admitted for the treatment of diarrhea to the Windhoek State Hospital in Namibia. No effort was made to obtain samples systematically. Stool specimens were stored at 4°C and were transported to the MRC Diarrhoeal Pathogens Research Unit, University of Limpopo Medunsa Campus, Pretoria, South Africa, for further analysis. After delivery, a 10% fecal suspension was prepared using distilled water, and the suspension was stored at 4°C.

Rotavirus antigens were detected using the 10% fecal suspensions and the commercially available Rotavirus IDEIA Kit (Oxoid), according to the manufacturer's instructions. All rotavirus-positive specimens were analyzed using polyacrylamide gel electrophoresis, as described elsewhere [13]. In brief, RNA was extracted from the 10% fecal suspensions with use of phenol-chloroform deproteinization and ethanol precipitation. The extracted RNA was eletrophoresed overnight through a 3% stacking and 10% resolving gel at 100V at ambient temperature with use of a discontinuous buffer system. The dsRNA bands were visualized using silver staining according to the method described by Herring et al [14]. Long and short electropherotypes were designated "L" and "S," respectively, and pattern numbers were arbitrarily assigned as new patterns were identified. All rotavirus-positive specimens were analyzed using an in-house VP6 subgroup (SG) enzyme-linked immunosorbent assay (ELISA), as described by Steele and Alexander [15]. Group-specific [16] and subgroup-specific monoclonal antibodies [17] were a kind donation from H. B. Greenberg, Stanford University (Stanford, CA).

VP7 serotyping was completed according to the method described by Taniguchi et al [18] on 53 rotavirus-positive specimens collected during 1998. An additional 6 specimens collected during 1998 were genotyped by reverse-transcription polymerase chain reaction (RT-PCR) methods described below. The following capture monoclonal antibodies were used: G1 [KU4], G2 [S2–2G10], G3 [YO-IE2], G4 [ST-2G7] [18] and G1 [5E8], G2 [IC10], and G3 [159] [19]. Mab60 [19] was also included as a control to detect the presence of the intact double-shelled viral particles. Each plate contained negative controls, and the reactions were read spectrophotometrically at 450 nm.

A total of 43 rotavirus-positive fecal suspensions collected

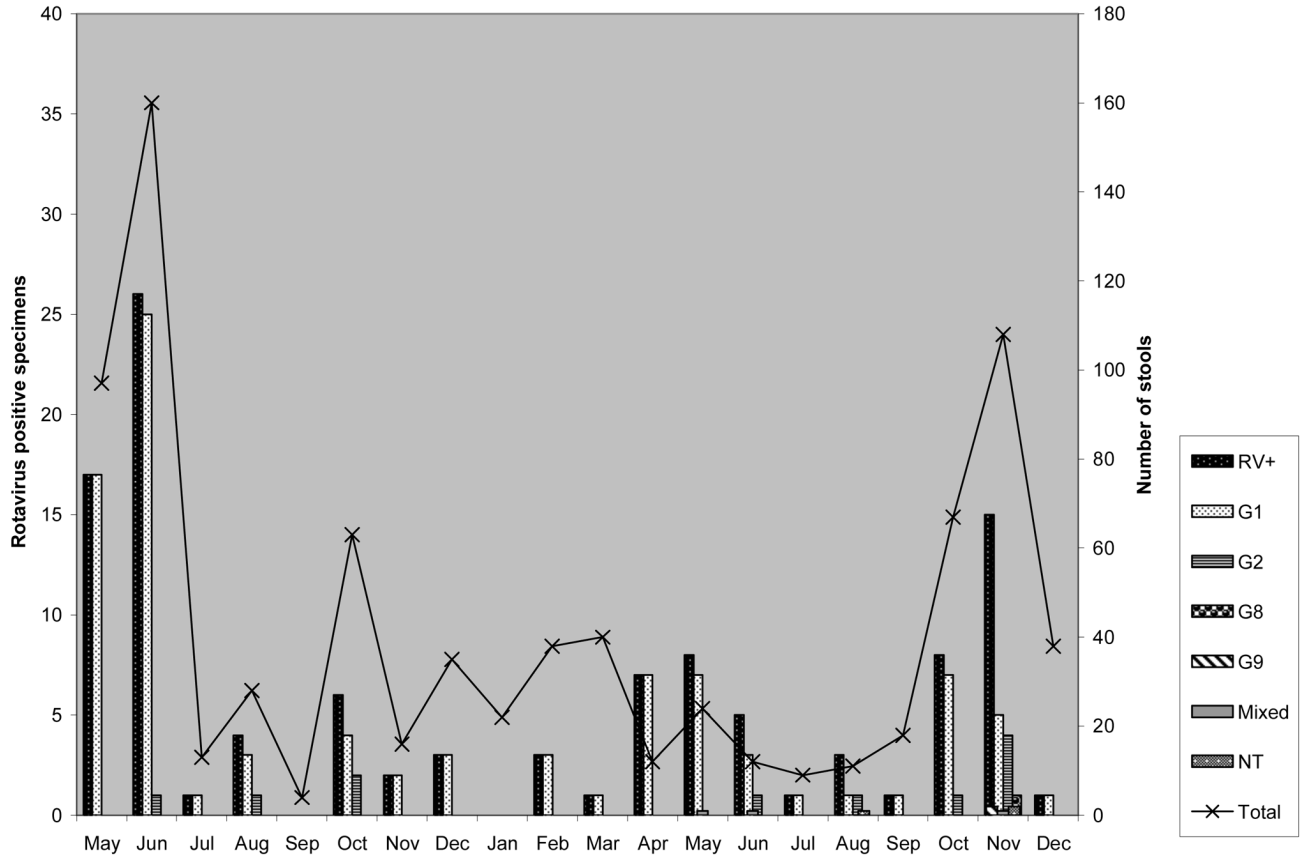

Figure 2. Monthly distribution of the total number of rotavirus-positive specimens ($n = 113$), numbers of various G serotypes and genotypes, and the total number of stool specimens collected from May 1998 through December 1999 at the Windhoek State Hospital in Namibia.

during 1998 were selected for further analysis of G type with use of RT-PCR. Specimens were selected to confirm the serotyping results, to determine the G genotype in specimens that could not be serotyped using monoclonal antibodies, and to determine G genotypes in specimens not previously analyzed. An additional 30 rotavirus-positive specimens were randomly selected for VP4 genotyping. RNA was extracted from the samples with use of a double extraction with phenol-chloroform (1:1), and the supernatant was mixed with 7M guanidine isothiocyanate, as described elsewhere [20]. The RNA was further purified using the RNAid kit (BIO 101), according to the manufacturer's instructions.

All of the rotavirus positive samples collected during 1999 were further analyzed using RT-PCR to determine G and P genotypes. Samples were extracted using the QIAamp Viral RNA extraction kit (Qiagen), according to the manufacturer's instructions, and were eluted in 60 μL TE buffer.

G and P genotypes were determined using the RT-PCR amplification methods developed by Gouvea et al [21] and Gentsch et al [22], respectively. The VP7- and VP4-specific genotyping primers used are described elsewhere [22–26].

RESULTS

From May 1998 through December 1998, 416 specimens were collected from children <5 years of age at the Windhoek State Hospital in Namibia. Rotavirus antigen was detected in 59 (14%) of these specimens. The following year, from January through December 1999, an additional 399 specimens were collected, and screening revealed an additional 54 rotavirus-positive specimens (13.5%). Overall, rotavirus was detected in 113 (13.8%) of 815 specimens obtained from the Windhoek State Hospital over the 20-month period. Analysis of the age distribution of rotavirus-positive specimens revealed that the majority of children (85%) affected were <18 months of age (Figure 1). Analysis of monthly distribution of rotavirus-positive specimens revealed that rotavirus season occurred mainly in autumn and winter, although a summer peak was evident in 1999 (Figure 2).

RNA migration patterns were determined for 94 (83%) of 113 specimens, and SG specificity was determined for 89 (79%) of 113 specimens (Table 1). Strains displaying the long L1 electropherotype and SGII specificity were predominant during both years of the study. However, the number of L2 strains

Table 1. Summary of the Subgroup Specificity and Electropherotypes in 113 Rotavirus-Positive Specimens Collected from May 1998 through December 1999 at the Windhoek State Hospital in Namibia

Year, electropherotype (subgroup specificity)	No. of specimens
1998	
L1 (II)	32
L1 (I/II)	1
L1 (non-I/non-II)	8
L1 (neg)	8
L2 (II)	4
L3 (neg)	1
S1 (I)	2
Neg (II)	1
Neg (non-I/non-II)	1
Neg (neg)	1
Total	59
1999	
L1 (II)	22
L1 (I)	1
L1 (neg)	3
L2 (II)	7
L2 (neg)	1
L4 (II)	1
S1 (I)	1
S2 (I)	2
Neg (II)	4
Neg (I/II)	1
Neg (I)	1
Neg (neg)	10
Total	54

NOTE. Long and short electropherotypes are designated "L" and "S," respectively, and pattern numbers were arbitrarily assigned. Neg, negative.

observed in 1999 was double that seen in the previous year. In addition, only 5 specimens with short electropherotypes were observed over the 20-month study period.

The results of G serotyping using monoclonal antibodies and RT-PCR and P genotyping using RT-PCR are summarized in Table 2. A total of 31 specimens (58%) were serotyped using an ELISA with serotype-specific monoclonal antibodies (data not shown). Fifteen specimens were typed using both monoclonal antibodies and RT-PCR genotyping methods, and the results were identical (data not shown). The remaining rotavirus-positive stool specimens were genotyped using heminested RT-PCR with serotype-specific primers. A total of 97 specimens were genotyped using G-specific primers, and 82 specimens were genotyped using P-specific primers (data not shown). Serotype G1P[8] strains predominated during the 20-month study, with lower levels of G1P[6], G2P[6], G1P[4], and G2P[4]; mixed G- and P-type infections were also detected (Table 2 and Figure 2). Serotype G8P[4] and G9P[6] strains were also found in 1 and 2 samples, respectively, during 1999.

DISCUSSION

From May 1998 through December 1999, 113 rotavirus-positive stool specimens were collected from the Windhoek State Hospital. During the 20-month study, serotype G1 strains predominated, accounting for 82% of all rotavirus strains detected in this area. G1 strains were found in combination with P[8], P[6], and P[4] genotypes in 30%, 12%, and 1.8% of specimens, respectively. Previous studies also detected G1 strains with P[8], P[6], and P[4] genotypes at levels of 19.3%, 2.9%, and 0.4%, respectively, in the African region and 52.2%, 0.6%, and 1.4%, respectively, in global surveys [9].

Serotype G2 was detected in 9% of strains identified in Namibia. Traditionally, serotype G2 strains are associated with short electropherotypes and SGI specificity. However, the Namibian G2 strains were unusual because they displayed both long electropherotypes with SGII specificity and short electropherotypes with SGI specificity. In 1998, 4 G2 strains were detected: 2 with SGI, short electropherotypes and 2 with SGII, long electropherotypes. The following year, 7 serotype G2 strains were detected: 4 with SGII, long electropherotypes; 2 with SGI, short electropherotypes; and 1 for which PAGE and SG results could not be determined. In addition to the unusual serotype G2 strains detected, 3 mixed G1/G2 infections with SGII, long electropherotypes were also observed. During the 1999 season, 1 serotype G8P[4] strain and 2 G9P[6] strains were detected. The G8 strain was associated with an SGI specificity and short electropherotype; of the 2 G9 strains, 1 had SGII specificity and a long profile, and the other had SGI specificity and a short profile.

In the relatively limited collection of Namibian stool spec-

Table 2. Summary of the G Serotyping and Genotyping and P Genotyping Results for 113 Rotavirus-Positive Specimens Collected from May 1998 through December 1999 at the Windhoek State Hospital in Namibia

	P type, no. of strains						
G type	P[4]	P[6]	P[8]	P[mix]	P[ND]	P[NT]	Total
G1	2	14	34	5[a]	32	6	93
G2	2	9	…	…	…	…	11
G8	1	…	…	…	…	…	1
G9	…	2	…	…	…	…	2
Gmixed	…	3[b]	…	…	…	…	3
GNT	…	1	1	…	…	1	3
Total	5	29	35	5	32	7	113

NOTE. Gmixed, G typing revealed >1 genotype; GNT, G genotype not typeable; P[mix], P typing revealed >1 genotype; P[ND], P typing not done; P[NT], P genotype not typeable.

[a] P mixed infection was four P[6]/P[8] and one P[4]/P[6].

[b] G mixed infection was G1/G2.

imens, a total of 12 (16.6%) of 72 fully genotyped specimens demonstrated evidence of reassortment (9 with G2P[6], 2 with G1P[4] 2, and 1 with G8P[4]). Detection and characterization of rotavirus strains from the United Kingdom during 1995–1999 demonstrated reassortment in only 2% of strains detected [27]. These results seem to indicate that reassortment among rotavirus strains in African settings is a common event and that these reassortants are ecologically fit and able to be transmitted.

The P[6] genotype has been identified in association with G1, G2, G8, and G9 strains at levels of 22.7% in the African region and 3% in global surveys [9]. In the Namibian study, P[6] was detected at slightly higher levels (25.6%) and was associated with G1, G2, and G9 strains. Serotype G9P[6] strains were detected during the early stages of G9 emergence globally [28–30], and Namibian G9 strains may have been a recent introduction into this region, although additional work needs to be conducted to confirm this observation. It has been suggested that the P[6] genotype may provide a mechanism for unusual or new rotavirus strains to enter a naive population, especially in Africa [31].

The 1998 rotavirus season in Windhoek occurred during the cool, dry winter months, showing a peak during May and June in a pattern similar to that seen in South Africa [32]. However, the 1999 season, although having the characteristic winter increase (albeit at lower levels), had a summer peak, with large numbers of rotavirus strains detected from October through December (Figure 2). Although the reduced winter peak and increased summer peak may be an artifact of stool collection, the summer rotavirus peak may indicate an outbreak in the Windhoek area. The appearance of rotavirus strains with greater diversity, including more mixed infections, and the appearance of new serotypes seems to support this idea.

Additional evidence sustaining this hypothesis reveals that the 1999–2001 rainfall seasons in southern Africa were dominated by active tropical cyclonic activity [33], and Namibia experienced heavy rainfalls in late 1999 [34]. The increased rainfall could also have contributed to the unusual summer peak in rotavirus infections and the increased genetic diversity seen in rotavirus strains, similar to the emergence dynamics observed in Bangladesh during the 1988 monsoon season [35]. In many African settings, increased rainfall in drier areas many contribute to the spread of fecal material in ground water and provide ample opportunities for multiple infections with various rotavirus serotypes.

The data provided here for Namibia have the potential to be the baseline for additional studies examining morbidity associated with rotavirus infection and impact on the local health care systems in the community. Although mortality associated with diarrheal disease in Namibia is 3%, the current burden of rotavirus disease is unknown. Updated information on the prevalence of rotavirus infection, circulating serotypes, and burden of disease is required to enable local government to make decisions regarding the implementation of rotavirus vaccination in this African setting.

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